Leishmania parasites. For example, the preparation may comprise the microfluidized lysate of *L. tropica*, *L. mexicana*, and *L. guyanensis*.

[39] The skin test assay of the present invention comprises administering to a subject an antigenic amount of a microfluidized lysate of the present invention. Preferably, the subject is a mammal, more preferably, the subject is human. Preferably, the microfluidized lysate preparation is administered intradermally, more preferably, the microfluidized lysate preparation is injected into the volar surface of the forearm of the subject. Induration is then measured. Preferably, induration is measured at least once at about 24 to about 72 hours after the microfluidized lysate preparation was administered. More preferably, induration is measured at about 48 hours after induration as true delayed type hypersensitivity responses in humans are maximal at about 48 hours. As used herein, an "antigenic amount" is an amount which provides a positive induration response in a subject known to be exposed to a leishmanial parasite. Antigenic amounts range from about 0.01 to about 1.0 ml, preferably about 0.05 to about 0.5 ml, more preferably about 0.1 ml. A positive response indicates previous exposure and sensitization to the antigen has occurred and is recalled, and an antigen specific inflammatory response takes place, thereby indicating that the subject was infected with or had been exposed to at least one *Leishmania* parasite. A positive response in humans is an induration of about 5 mm or more.

[40] As explained in Example 3, the microfluidized lysate preparations of the present invention are safe and immunogenic. Therefore, the present invention also provides immunogenic compositions and vaccines comprising the microfluidized preparations of the present invention. The vaccine or immunogenic compositions of the present invention may be used in combination with an adjuvant, a pharmaceutically acceptable excipient, or both. Thus, the present invention also provides methods for immunizing subjects against diseases, infections, or disorders associated with *Leishmania* parasites, such as Leishmaniasis, all forms of leishmaniases, and diseases as Chagas Disease, and African trypanosomiasis were common crossreacting antigens with immunoprotective properties.

[41] Since the method of preparing the microfluidized lysate preparations as described herein may be standardized and reproduced to prepare batches of microfluidized lysate preparations having substantially similar characteristics, such as potency and specificity, the microfluidized lysate preparations may be used to purify or screen for ligands which

bind to leishmanial antigens or antibodies raised against the microfluidized lysate of the present invention with consistency.

[42] Additionally, the microfluidized lysate preparations of the present invention are suitable for use as immunogens to raise anti-leishmanial antibodies. The antibodies may be prepared by immunizing a suitable subject, *e.g.*, rabbit, goat, mouse or other mammal, with the microfluidized lysate of the present invention by methods standard in the art. The microfluidized lysate preparation may further include an adjuvant, such as Freund's complete or incomplete adjuvant, or a similar immunostimulatory agent. Accordingly, the present invention also provides antibody preparations made by immunizing a suitable subject with a microfluidized lysate preparation of the present invention. The antibodies produced by the subject may be isolated or purified by methods standard in the art.

[43] The antibodies raised against the microfluidized lysate of the present invention may be used to isolate leishmanial antigens by methods standard in the art, such as affinity chromatography or immunoprecipitation. The antibodies raised against the microfluidized lysate of the present invention may be used to diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable label or marker. Examples of detectable labels and markers include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

[44] The microfluidized lysate preparation, antibodies, or antigens of the present invention may be incorporated into a pharmaceutical composition suitable for administration. Such compositions typically comprise the microfluidized lysate preparation, antibodies, or antigens of the present invention and a pharmaceutically acceptable carrier. Preferably, pharmaceutical compositions of the present invention comprise an antigenic amount of the microfluidized lysate preparation or antigen or a

therapeutically effective amount of antibodies raised against the microfluidized lysate preparation, and an inert, pharmaceutically acceptable carrier or diluent. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Preferably, the pharmaceutical compositions of the present invention include sterile saline comprising 0.4% phenol.

[45] The antigenic activity of the microfluidized lysate of the present invention may be measured by any of the methods available to those skilled in the art, including *in vitro* and *in vivo* assays. Examples of suitable assays for activity measurements are provided herein. Properties of the microfluidized lysate, such as protein content, endotoxin content, pH, sterility, purity, and color, may be assessed, for example, by methods standard in the art. Other pharmacological methods may also be used to determine the efficacy of the microfluidized lysate as antigenic compositions.

[46] The following examples are intended to illustrate but not to limit the invention.

Example 1

Process for Making *L. guyanensis* Microfluidized Lysate

[47] Source material and production of a research seed for the *L. guyanensis* Leishmania Skin Test, LSTA -Lg (BPR-2334-RS) was conducted as follows. A parasite specimen was obtained from an active dermal lesion from a human subject otherwise healthy with unremarkable medical history. The subject tested negative in screening with viral/STD panel (HIV, HTLV, CMV, HCV, HBV and syphilis). The subject's travel history was determined.

[48] The absence of adventitious agents in all media components used in manufacturing process was determined and (The Fetal Bovine Serum (FBS) Type II, was mycoplasma and bovine virus tested, screened for bacteriophage, tested for endotoxin, tested for AVA, cell culture tested, heat-inactivated, and from a non BSE country (Gibco Life Technologies, Grand Island, NY; Fetal Bovine Serum, Qualified, Origin-United States, Catalogue Number 26140, Lot Number 1016982). The promastigotes cultured from this specimen, (strain WR2334), were determined by isoenzymes to be *L.*